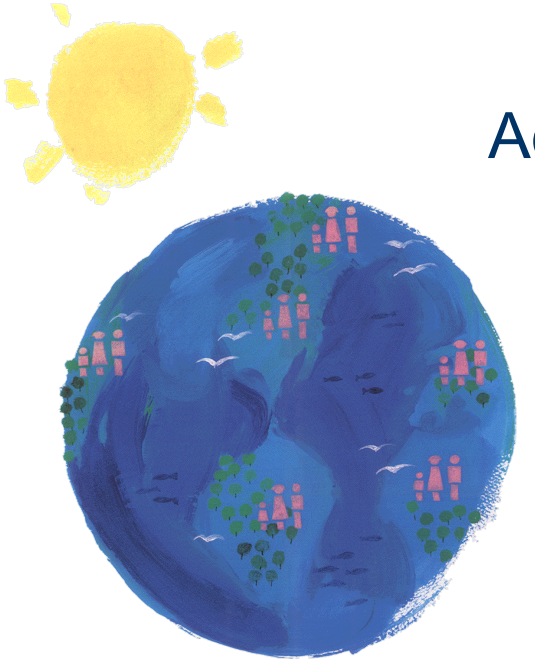
NICEATM — ***National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods***

ICCVAM — ***Interagency Coordinating Committee on the Validation of Alternative Methods***



Upcoming Leptospira Vaccine and Acellular Pertussis Vaccine Workshops

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Co-Chairs, ICCVAM Biologics Working Group

SACATM Meeting
September 6, 2012
National Institute of Environmental Health Sciences
Research Triangle Park, NC

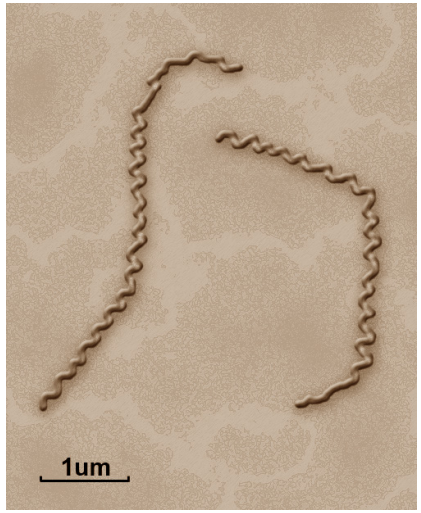
National Toxicology Program
U.S. Department of Health and Human Services

Workshop on Alternative Methods for Leptospira Vaccine Potency Testing

- September 19-21, 2012
- U.S. Department of Agriculture Center for Veterinary Biologics National Centers for Animal Health
  Ames, Iowa, USA
- Experts from government, academia and industry
- Plenary and Breakout Sessions
- Poster Session
- Workshop proceedings to be published in *Biologicals*

Further information is available at:
http://iccvam.niehs.nih.gov/restrict/leptowksp/leptowksp.htm

Leptospirosis



- Bacterial zoonotic disease caused by spirochetes of the genus Leptospira with a worldwide distribution
- Leptospira has greater than 200 pathogenic serovars, and divides into 25 serogroups
- More than 500,000 human cases of severe leptospirosis occur worldwide each year with a fatality rate of 1% – 25%
- There are no widely used human Leptospiral vaccines; disease is controlled by antibiotic treatment
- Mainly a disease of dogs, cattle, sheep, goats and swine.
- Vaccines have been available for use in animals for several decades
  - Typically 4-5 serovars often in combination with other viral or bacterial vaccines
- Potency testing requires large numbers of laboratory animals (hamsters)
  - Many experience significant unrelieved pain and distress

Leptospira spp. Vaccine Potency Testing: ICCVAM Priority Activity

- At the September 2010 workshop[1], *Leptospiral spp.* vaccines were identified as one of the three highest priorities for future research, development, and validation efforts because:
  - Many serials are produced annually for use in multiple veterinary species
  - Potency tests use large numbers of laboratory animals
    - 48,015 hamsters (pain and no drug used) – all testing (2010)[2]
    - 38,412 (80%) are estimated to be used in vaccine testing
  - Challenge test involves significant unrelieved pain and distress
  - Vaccine challenge tests requiring live bacteria are hazardous to laboratory workers
  - 3Rs implementation of humane endpoints and *in vitro* ELISA development for Leptospira potency testing has resulted in refinement and replacement of hamster usage
    - ~45,000 in 2002 to ~33,000 in 2009[3]

[1]International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing: State of the Science and Future Directions, September 14-16, 2010, Bethesda, MD, USA.
[2]USDA 2010 Annual Report Animal Usage.
[3]Kulpa-Eddy, J. Development of *in vitro* Leptospira Potency Test. Langen, Germany. December 1-3, 2010.

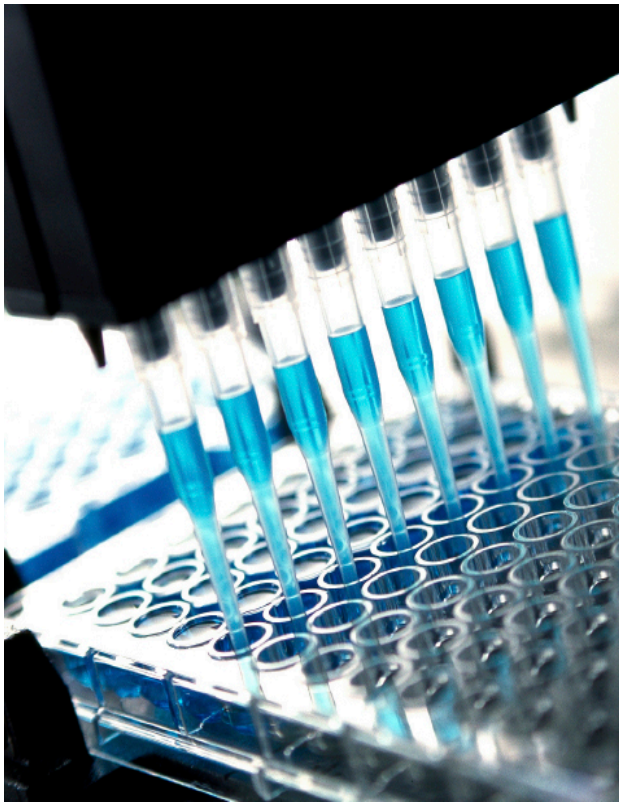
Leptospira Vaccine Potency Testing: Refinement Methods

- Serology
  - Ph.Eur. Monograph 447 (Multi-component Canine Leptospirosis vaccine) and Ph.Eur. Monograph 1939 (Multi-component Bovine Leptospirosis vaccine)
    - Last revision 01/2008

Leptospira Vaccine Potency Testing: Replacement Methods

Replacement Methods - *In vitro*

- 1990-2000 - ELISA methods developed by the USDA in the 1990's and Supplemental Assay Methods (SAMs) published in 2000
- 2005-2009 - CVB validated the Reference vaccines in the host animal (dogs, pigs) and correlated to the hamster challenge model. ELISA assays were then correlated to the hamster challenge model.
- *In vitro* ELISAs for *Leptospira interrogans* serovars *pomona*, *canicola*, *grippotyphosa* and *icterohaemorrhagiae*



Importance of Leptospira Workshop

- If replacement and refinement methods have been written into the regulatory guidance documents, why is this workshop necessary?
  - Implementation issues based on product specific validation
    - Presence of adjuvant
    - Correlation to target animal efficacy and/or hamster potency
    - Qualification and testing of references
  - Necessary to bring all stakeholders together in a non-regulatory setting.
  - International communication is critical - pre-workshop planning teleconferences are identifying, clarifying and prioritizing key topics for discussion at the workshop

Leptospira Workshop Objectives

- Animal and Public Health Perspectives
  - Identify and review public health needs
  - Regulatory requirements for potency testing of *Leptospira* vaccines
- State of the Science
  - Review the state of the science of currently available alternative methods for *Leptospira* vaccine potency testing
- Implementation
  - Identify any unresolved data gaps and develop an implementation strategy to achieve global regulatory acceptance of alternative methods
- Future considerations
  - Identify best practices for current and future integrated approaches to *Leptospira* vaccine potency testing

Acknowledgements:
ICCVAM Interagency Biologics Working Group

U.S. Food and Drug Administration

Center for Biologics Evaluation and Research

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Center for Drug Evaluation and Research

- Abigail Jacobs, PhD (CDER)

Center for Food Safety and Nutrition

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- Hans Draayer, MSc
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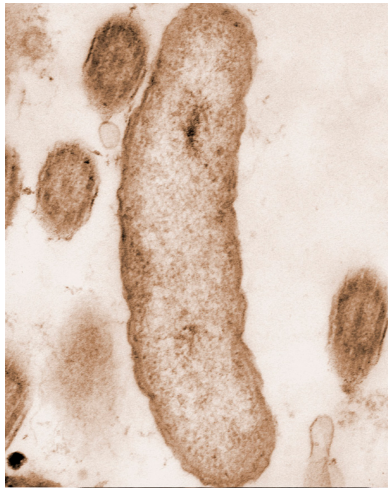
  USA

Workshop on Alternatives to the Murine Histamine Sensitization Test (HIST) for Acellular Pertussis Vaccines

- November 28-29, 2012
- William H. Natcher Conference Center
  National Institutes of Health,
  Bethesda, MD
- Experts from government, academia and industry expected to attend
- Plenary and Breakout Group Sessions
- Poster Session
- NICEATM coordinating with the International working group on alternatives to HIST
- Review of *in vitro* safety data on spiked Pertussis toxin vaccine preparations
- Workshop report to be published

Further information is available at:
http://iccvam.niehs.nih.gov/meetings/HISTWksp-2012/HISTWksp.htm

Pertussis (Whooping Cough)



- Highly contagious disease caused by the bacterium *Bordetella pertussis* and characterized by violent coughing
- Whole cell vaccine introduced in the 1940s
  - Replaced by an acellular vaccine over the last 20 years
- Periodic epidemics every 3 to 5 years and frequent outbreaks
  - During past 5 years, 10,000 to 27,000 cases reported annually in the US
- Murine HIST is a key safety test performed to assay for residual active pertussis toxin prior to vaccine release
  - Based on the sensitization to histamine induced by active pertussis toxin
  - Requires large numbers of laboratory animals (mice) that experience unrelieved pain and distress

Pertussis Vaccine Safety Testing: Priority Activity (1)

- At the September 2010 workshop[1], Pertussis vaccines were identified as one of the three highest priorities for human vaccines for future research, development, and validation efforts because:
  - Many lots are produced annually
  - HIST use large numbers of laboratory animals
  - HIST involves significant unrelieved pain and distress in mice
  - HIST is highly variable often requiring frequent repeats

Pertussis Vaccine Safety Testing: Priority Activity (2)

- Previous HIST workshops[1,2] established an International Working Group on Alternatives to HIST for testing alternative *in vitro* methods using standardized acellular pertussis vaccines and pertussis toxin
  - 12 international laboratories involved
  - 7 vaccines from 3 manufacturers (GlaxoSmithKline, Sanofi Pasteur, Statens Serum Institute)
  - Standardized pertussis toxin spiking protocol
  - Study to be conducted Summer 2012

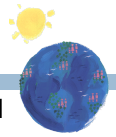
[1]Workshop on Animal-Free Detection of PTx in Vaccines – Alternatives to HIST, PEI, Langen, Germany, June 9-10, 2011.
[2]Alternative Safety Testing Strategies for Acellular Pertussis Vaccines (8th World Congress Satellite meeting), Montreal, Canada, August 21, 2011

Pertussis Workshop Objectives (1)

- Review the usefulness and limitations for alternative *in vitro* test methods proposed to replace the current *in vivo* HIST
- Review *in vitro* protocols and data generated by participants of the International Working Group on Alternatives to HIST.
  - Use of common set of vaccines, pertussis toxin (reference standard), and protocol for spiking
  - *In vitro* assays tested
    - Biochemical assays
      - Binding assay: used to assess the amount of pertussis toxin/toxoid binding activity to the glycoprotein fetuin.
      - Enzymatic assay: monitors the residual ADP-ribosylation of the pertussis toxin/toxoid
    - Cell-based assays
      - Human cells measuring ATP reduction
      - Rat cells measuring cAMP
      - CHO cell (morphological, cytopathic)

Pertussis Workshop Objectives (2)

- Discuss application of these *in vitro* assays for monitoring consistency of vaccine manufacture as alternatives to the HIST
- Establish framework for international collaboration to validate *in vitro* assay(s) for acellular pertussis vaccine testing
- Identify regulatory acceptance requirements for *in vitro* assays as alternatives to the HIST



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